REMARKS

Reconsideration and withdrawal of the rejections of the application are respectfully requested in view of the remarks and attachments herein.

The September 13, 2005 Office Action stated that claims 14-22 remain rejected under 35 U.S.C. §112, first paragraph because the specification allegedly does not provide enablement for all compounds which inhibit the reductase activity of 11-Beta-hydroxysteroid dehydrogenase in adipose tissue. Applicants respectfully traverse.

Specifically, the Office Action states that only one compound is identified in the specification that could be used by the skilled artisan to practice the invention and that other such compounds are lacking. In addition, the Office Action stated that the Declarations of Brian R. Walker and Jonathan R. Seckl had been considered, but were not sufficient to demonstrate that numerous compounds that are known inhibitors of 11 beta-hydroxysteroid dehydrogenase are well known to those of skill in the art. Applicants respectfully disagree with this statement and request that the Examiner reevaluate his stance on the declarations, in view, *inter alia*, of his reliance on the declarations in related and copending application U.S.S.N. 10/080,875.

For instance, U.S.S.N. 10/080,875, was subject to similar enablement rejections which stated that the specification described a single compound, and that knowledge of other such compounds was lacking in the specification and to those of skill in the art. Applicants filed a Declaration by Brian R. Walker and Jonathan R. Seckl, which stated that numerous compounds that are known inhibitors of 11 beta-hydroxysteroid dehydrogenase are well known to those of skill in the art, and which was identical to the Declaration filed in the instant application in the substantive statements therein, the filing of which resulted in withdrawal of the enablement rejections under 35 U.S.C. §112 in U.S.S.N. 10/080,875.

The September 13, 2004 Office Action discounts the Declaration of Walker and Seckl by stating that the claims are drawn to a method of reducing circulating fatty acids in adipose tissue in a patient who can be suffering from obesity, insulin resistance, or both, and that Monder and White demonstrate the different compounds have different activity in various tissues, such that undue experimentation would be required to determine which of the compounds would have the desired effect on the specific tissue. Applicants respectfully request that the Examiner review Monder and White, wherein it states "the **magnitude** of the inhibitory effects of steroid analogs differs between tissues". Monder and White, page 199, second full paragraph. Therefore, in

contrast to what is intimated in the Office Action, the compounds do not different types of activity in various tissues. Rather, there is a difference in the magnitude of the activity of the compounds in various tissues, not in the type of activity. As any therapeutic compound must undergo basic, standard testing to determine the most effective dose, it is respectfully submitted that any testing of compounds that are known inhibitors of 11 beta-hydroxysteroid dehydrogenase need only rise to such a basic level that it cannot be considered undue experimentation.

Additionally, the Office Action stated that Monder and White confirmed that not all inhibitors are known to act on various tissues effectively in stating "[s]teroids devoid of oxygen at C-11 are generally not inhibitors, or inhibit oxidation poorly." To the contrary, this statement in Monder and White instead provides guidance to those of skill in the art while selecting a compound for use in the practice of the present invention; that is, one of skill in the art would recognize this class of unsuitable compounds and would not select one of these compounds.

Furthermore, the Examiner's attention is respectfully drawn to the attached documents Alberts (2002), Alberts (2003) and the attached abstracts from the 23rd Joint Meeting of the British Endocrine Sciences and from the North American Association for Study of Obesity. These documents, along with WO2004/033427, WO2004/041264, WO2004011410, WO2003/104207, WO2003/104208, WO2003/065983, WO2004/058741, WO2004/037251, WO2004/056745, WO2004/089470, WO2004/089415, WO2004/089367, WO2004/089380, WO2004/089471, WO2004/089896 and WO2004/065351, are indicative of the post-published art that shows further inhibitors could be easily identified by the skilled artisan; and therefore that the invention is and was enabled for all inhibitors of HSD1.

Accordingly, as the Declaration of Walker and Seckl and the documents attached thereto, including Monder and White, demonstrate that one of skill in the art had knowledge of suitable compounds for use in the practice of the present invention, would readily be able to determine other effective compounds, and would not have to engage in undue experimentation to do so, it is respectfully submitted that the present claims are fully enabled. Consequently, reconsideration and withdrawal of the rejections under 35 U.S.C. §112, first paragraph, is respectfully requested.

REQUEST FOR INTERVIEW

If any issue remains as an impediment to allowance, an interview with the Examiner is

respectfully requested, prior to issuance of any paper other than a Notice of Allowance; and, the Examiner is respectfully requested to contact the undersigned to arrange a mutually convenient time and manner for such an interview.

CONCLUSION

In view of the amendments and remarks herewith, it is respectfully submitted that the application is now in condition for allowance. Favorable reconsideration of the application and prompt issuance of a Notice of Allowance, or an interview at a very early date with a view to placing the application in condition for allowance, are earnestly solicited. The undersigned looks forward to hearing favorably from the Examiner at an early date.

Respectfully submitted,
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Diabetologia

Selective inhibition of 11β-hydroxysteroid dehydrogenase type 1 decreases blood glucose concentrations in hyperglycaemic mice

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Abstract

Aims/hypothesis. Current pharmacological treatments for Type II (non-insulin-dependent) diabetes mellitus have various limitations. New treatments are needed to reduce long-term risks for diabetic complications and mortality. We tested a new principle for lowering blood glucose. It is well known that glucocorticoids in excess cause glucose intolerance and insulin resistance. The enzymes 11β-hydroxysteroid dehydrogenase type 1 and type 2 inter-convert inactive and active glucocorticoids, thereby playing a major role in local modulation of agonist concentration and activation of corticosteroid receptors in target tissues. It has been hypothesized that selective inhibition of 11β-hydroxysteroid dehydrogenase type 1 decreases excessive hepatic glucose production in hyperglycemia and diabetes. BVT.2733 is a new, small molecule, non-steroidal, isoform-selective inhibitor of mouse 11β-hydroxysteroid dehydrogenase type 1. The aim of the present study is to test if selective inhibition of 11β-hydroxysteroid dehydrogenase type 1 lowers blood glucose concentrations in a hyperglycaemic and hyperinsulinaemic mouse model.

Methods. BVT.2733 was given to spontaneously hyperglycaemic KKA^y mice for 7 days using subcutaneous osmotic mini-pumps.

Results. BVT.2733 lowered hepatic PEPCK and glucose-6-phosphatase mRNA, blood glucose and serum insulin concentrations compared with vehicle treated mice. In contrast, hepatic 11β-hydroxysteroid dehydrogenase type 1 mRNA, liver function marker enzyme expression (aspartate aminotransferase, alanine aminotransferase and alkaline phosphatases), daily food intake and body weight were not altered by the treatment.

Conclusion/interpretation. These results suggest that a selective inhibitor of human 11β-hydroxysteroid dehydrogenase type 1 can become a new approach for lowering blood glucose concentrations in Type II diabetes. [Diabetologia (2002) 45:1528–1532]

Keywords Hydroxysteroid dehydrogenases, 11β-HSD1, blood glucose, gluconeogenesis, glucose-6-phosphatase, hyperglycaemia, phosphoenolpyruvate carboxykinase (GTP), pharmacology, oral pharmacotherapy, diabetes.

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Abbreviations: 11,; β ,; -HSD1, 11β -hydroxysteroid dehydrogenase type 1; ALP, alkaline phosphatases; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BVT.2733, 3-chloro-2-methyl-N-{4-[2-(4-methyl-1-piperazinyl)-2-oxoethyl]-1,3-thiazol-2-yl} benzenesulfonamide; FAM, 6-carboxyfluorescein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; G6Pase, glucose-6-phosphatase; PBS, phosphate buffered saline; TAMRA, 6-carboxytetramethylrhodamine.

The liver is central in glucose homeostasis and plays a major role in the aetiology of glucose intolerance and Type II (non-insulin-dependent) diabetes mellitus. Under normal conditions gluconeogenesis, that is the synthesis of glucose from lactate, pyruvate, glycerol and glucogenic amino acids accounts for approximately 25% of endogenous hepatic glucose production, the rest being due to glycogenolysis [1]. However, in Type II diabetes as much as 90% of the hepatic glucose output can be due to accelerated gluconeogenesis [1]. Glucocorticoids are potent functional antagonists of insulin action and are essential for increased hepatic glucose output and raised blood glucose concentrations in

diabetes [2, 3, 4, 5]. Glucocorticoids are involved in the transcriptional control of several genes involved in the regulation of hepatic glucose production, including *PEPCK*, encoding the enzyme catalysing the rate-limiting step in gluconeogenesis [6].

Tissue response to glucocorticoids is regulated by the glucocorticoid receptor and intracellular synthesis of active glucocorticoids by 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1; EC 1.1.1.146). In humans, 11β-HSD1 converts cortisone into active cortisol [7, 8, 9, 10, 11]. In rodents, the inactive glucocorticoid is 11-dehydrocorticosterone and the active is corticosterone. *11β-HSD1* is widely distributed and is highly expressed in human liver and is also found in adipose tissue [10, 12] and pancreatic beta cells [13]. Another enzyme, 11β-HSD type 2 catalyses the reverse reaction and is found for example in kidney and placenta [14, 15, 16]. Inhibition of the type 2 enzyme in the kidney results in serious conditions associated with inappropriate mineralocorticoid receptor activation by glucocorticoids, like sodium retention, hypokalaemia and hypertension [10, 17].

11β-HSD1 alters glucocorticoid hormone action in target tissues for insulin action and has been suggested to play a regulatory role in glucose homeostasis. In a clinical study, a non-selective 11β-HSD1 inhibitor has been shown to enhance insulin sensitivity in healthy subjects [18]. Moreover, studies done in the *11β-HSD1* gene knock-out mice suggest that enzyme inhibition could decrease blood glucose concentrations without risk for hypoglycaemia [19]. Both fasting blood glucose concentrations and hepatic transcription of PEPCK and glucose-6-phosphatase (G6Pase) mRNA were lower in the *11β-HSD1* gene knock-out mice compared with wild-type mice [19]. In contrast, overexpression of the *11β-HSD1* gene in adipose tissue caused hyperglycaemia, glucose intolerance, hyperinsulinaemia, insulin resistance, and visceral obesity [20].

Due to lack of specific 11β-HSD1 inhibitors [21], non-selective inhibitors of both 11β-HSD enzymes type 1 and type 2, including glycyrrhizic acid, glycyrrhetinic acid and carbenoxolone, have been used in previous attempts to assess the functional role of 11β-HSD1 in glucose homeostasis [18]. BVT.2733 is a new type 1 selective inhibitor of murine 11β-HSD1 (K_i 1 μmol/l, using 11-dehydrocorticosterone as substrate), which does not inhibit mouse 11β-HSD type 2 at a concentration as high as 200 μmol/l (corresponding to a K_i>33 μmol/l, using 50 nmol/l corticosterone as substrate; R. Olsson, personal communication). Thus, the selectivity of BVT.2733 for the 11β-HSD type 1 enzyme over the type 2 enzyme is estimated to be at least 30-fold based on the K_i values. Our experiments focused on investigating the effects of inhibition of hepatic 11β-HSD1 on glycaemic control in hyperglycaemic mice.

Materials and methods

Animals. Male KKA^y mice, age 6 to 12 weeks (Clea, Tokyo, Japan), were housed individually at 22±1°C, 12:12 h light-to-dark cycle, and fed a high-fat diet (32.5 kcal% fat, D12266B; Research Diets, New Brunswick, N.J., USA) and water ad libitum at least 3 weeks before the experiments. Animals were grouped according to 4-h fasting blood glucose concentrations. The procedures involving animals were in conformity with national and international laws for the care and use of laboratory animals. The local animal ethics committee approved the experiments.

Animals were anaesthetized with enflurane (Efrane, Abbot Scandinavia, Solna, Sweden). Alzet (Alza, Palo Alto, Calif., USA) osmotic mini-pumps (model 2001; 0.2 ml, pumping rate 1.0 μl/h) were inserted subcutaneously (s.c.) on the back of the neck. In a separate experiment, hepatic 11β-HSD1 inhibition was measured after oral BVT.2733 administration.

Hepatic 11β-HSD1 activity. Snap frozen liver lobes (0.5–2 g) in liquid nitrogen, stored at −70°C, were homogenized (0.2 g/ml) in PBS (mmol/l: NaCl 136.9, Na_2HPO_4 8.1, KCl 2.7, KH_2PO_4 1.3, pH 7.5; SVA, Uppsala, Sweden) and incubated with [1,2(n)]-^{3}H-cortisone (33 nmol/l) (Amersham Pharmacia Biotech, Uppsala, Sweden) and NADPH (33 μmol/l) for 15 min (37°C; Tris 30 mmol/l, pH 7.2, EDTA 1 mmol/l). The reaction was stopped with perchloric acid and the samples were centrifuged at 1300 g for 3 min. ^{3}H-Cortisol and ^{3}H-cortisone in the supernatant were separated by HPLC (HP1100; Hewlett-Packard, Palo Alto, Calif., USA; 15 μl injection volume, 5 μm diameter C18 column; acetonitrile:water 28:72, 0.8 ml/min) [22]. Radioactivity was measured by adding scintillation cocktail (Ultima-Flo, Packard, Meriden, Conn., USA) to the effluent and passed through an on-line liquid scintillation spectrometer (Flow scintillation analyser, Packard).

Hepatic mRNA. Real-time PCR was used to quantify mRNA concentrations of 11β-HSD1, PEPCK and G6Pase (TaqMan, Applied Biosystems, Foster City, Calif., USA). Total RNA was prepared from frozen livers using RNAqueous (Ambion, Tex., USA) and treated with DNase (Ambion). The cDNA was prepared from all of the total RNA samples using the TaqMan reverse transcription reagent. The PCR was made with primer, probe, TaqMan Universal PCR Master Mix, and cDNA (each sample in triplicate). The ABI Prism 7700 Sequence Detection System (software version 1.6) was used for analysis. Results were normalized to endogenous control GAPDH mRNA concentrations (Applied Biosystems). GAPDH mRNA concentrations were compared with 18S rRNA and did not differ between groups.

In each case the amplicon includes a sequence corresponding to an exon border in the genome. The *11β-HSD1* probe and the forward primers for *PEPCK* and *G6Pase* span exon borders in the respective genes.

The following forward primers, reverse primers, and probes, respectively, were designed (melting temperature,°C; concentration, nmol/l):

11β-HSD1:

5′-agcagagcaatggcagcat (58, 300);
5′-gagcaatcataggctgggtca (59, 50);
5′FAM-cgtcatctcctccttggctgggaa-TAMRA (68, 100).

PEPCK:

5′-ggcggagcatatgctgatcc (61, 500);
5′-ccacaggcactagggaaggc (60, 50);
5′FAM-ccccgaaggcaagaagaaatacctggc-TAMRA (70, 200).

G6Pase:

5'-tcaacctcgtcttcaagtggatt (59, 300);
5'-gctgtagtagtcggtgtccagga (59, 300);
5'FAM-tgtttggacaacgcccgtattggtg-TAMRA (69, 100).

Blood chemistry. Glucose concentration was measured immediately upon sampling (Accutrend, Roche, Basle, Switzerland). The mice were not fasted during the experiment, since fasting induces hepatic PEPCK mRNA transcription [19].

Serum was prepared from blood obtained after cervical dislocation and left half an hour at 4°C before centrifugation for 10 to 15 min at 3000 g. The serum was stored at −70°C until analysis.

Serum insulin was analysed with rat insulin RIA (Linco, St. Louis, Mo., USA). Serum AST, ALT and ALP activity was measured with kinetic UV methods (Roche).

Serum and hepatic BVT.2733. Serum (10 µl) was diluted with 200 µl trifluoroacetic acid (1%) containing internal standard (carbamazepine; 0.05 µmol/l). Fifty microlitres of the diluted serum was injected on a Prospect on-line solid phase extraction system (Spark Holland, Emmen, The Netherlands) loaded with extraction cartridges (Waters Oasis, Milford, Mass., USA; 10*2 mm I.D.) coupled to liquid chromatography – tandem mass spectrometry detection (LC-MS-MS; Micromass Quattro II, Manchester, UK).

Liver samples were homogenized in PBS (0.2 g/ml). Proteins were precipitated from the homogenate (0.1 ml) by acetonitrile (0.4 ml) with internal standard (carbamazepine; 0.5 µmol/l). After 30 min at room temperature the samples were centrifuged for 5 min (18200 g), the supernatant (50 µl) was diluted with 150 µl trifluroacetic acid (1%), and 50 µl of the dilute was injected on the solid phase extraction system coupled to LC-MS-MS as described for the serum samples.

The instrument was set up with an auto-injector (Gilson ASPEC XL, Middleton, Wiss., USA) and LC-pumps (Shimadzu 10AD, Kyoto, Japan). The analytes were separated on an HPLC-column (Zorbax SB-18, 2.1*50 mm, 5 µm; Agilent Technologies, Wilmington, Del., USA). The mobile phase consisted of (i) 2% acetonitrile and 98% formic acid (8 mmol/l), and (ii) 95% acetonitrile and 5% formic acid (133 mmol/l). The flow rate was 0.3 ml/min with a mobile phase gradient from 10 to 100% of (B) over a period of 3 min. Detection was achieved by electrospray in positive ion mode and multiple reaction monitoring for the transition in mass per charge 429 to 140 for BVT.2733 and mass per charge 237 to 194 for carbamazepine. BVT.2733 (serum: 0.05–50 µmol/l; liver: 1–100 µmol/l) standards were made in mouse serum or in pooled mouse liver homogenate (vehicle).

Food intake and body weight. Animals and fodder were weighed for 3 days before and every morning after pump implantation.

Compound. 3-chloro-2-methyl-N-{4-[2-(4-methyl-1-piperazinyl)-2-oxoethyl]-1,3-thiazol-2-yl} benzenesulfonamide (BVT.2733) (Biovitrum, Stockholm, Sweden) was dissolved in aqueous (2-β-hydroxypropyl)-cyclodextrin (12%; Fluka, Steinheim, Germany).

Statistics. The Mann-Whitney non-parametric test (SPSS 10.1, Chicago, IL., USA) was used for statistical evaluation of the results. Results are expressed as means ± SEM.

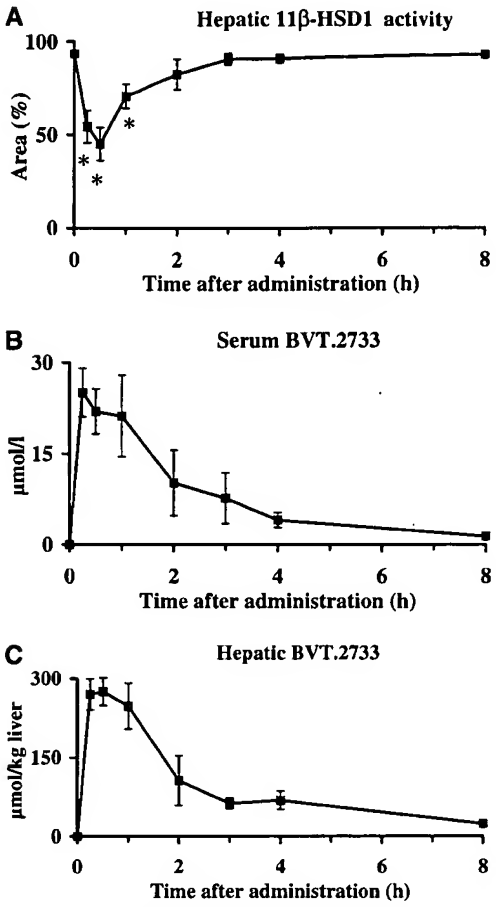

Fig. 1A–C. Effect of a single BVT.2733 (100 mg·kg^{-1}; p.o.; n=4) administration in KKA^{y} mice. **A** Hepatic 11β-HSD1 activity (^{3}H-cortisol (^{3}H-cortisone+^{3}H-cortisol)$^{-1}$) (Area, %). **B** Serum BVT.2733 concentrations (µmol/l). **C** Hepatic BVT.2733 levels (µmol/kg liver). Statistical difference (p) from control is: *p<0.05

Results

Acute administration. Administration of a single bolus dose of BVT.2733 (100 mg·kg^{-1}; p.o.) resulted in an inhibition of hepatic 11β-HSD1 activity (Fig. 1A). The maximal inhibition of hepatic 11β-HSD1 activity occurred after approximately half an hour and correlated with the maximum concentration of BVT.2733 both in liver and blood (Fig. 1B, C).

7-Day administration. BVT.2733 was administered as a continuous subcutaneous infusion by osmotic minipumps (167 mg·kg^{-1}·day^{-1}). After 7 days of treatment, serum BVT.2733 concentration was 7.4±2.6 µmol/l (n=22) and liver concentrations were 24.1± 6.2 µmol/kg (n=10). At that time, the hepatic PEPCK mRNA concentration was decreased to 75±17% (n=10; p<0.05) and G6Pase mRNA concentration to 55±51% (n=10; p=0.089) of the concentrations in vehicle treated animals (Fig. 2). However, hepatic 11β-HSD1 mRNA concentration was not altered

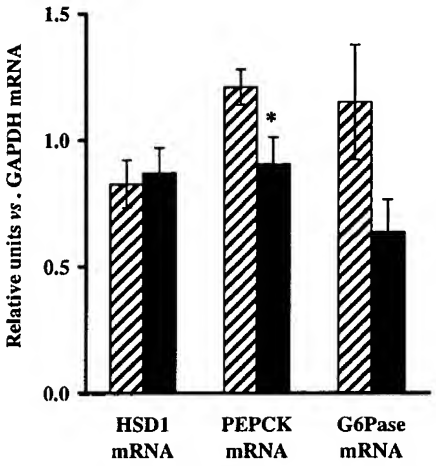

Fig. 2 Effect of 7-day BVT.2733 (167 mg·kg^{-1}·day^{-1}; s.c. osmotic pump; n=10) administration in KKA^{y} mice on hepatic mRNA concentrations of 11β-HSD1, PEPCK and G6Pase. Results are given as relative units vs GAPDH mRNA. Statistical difference (p) from vehicle is: *p<0.05. (*hatched bars*) Vehicle and (*solild bars*) BVT.2733

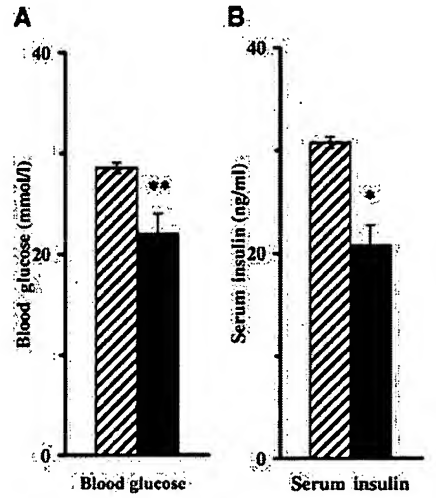

Fig. 3A, B. Effect of 7-day BVT.2733 (167 mg·kg^{-1}·day^{-1}; s.c. osmotic pump) administration in KKA^{y} mice. **A** Blood glucose concentrations (BVT.2733: n=17, vehicle: n=15). **B** Serum insulin concentrations (BVT.2733: n=19, vehicle: n=20). Statistical difference (p) from vehicle is: *p<0.05, **p<0.01. (*hatched bars*) Vehicle and (*solid bars*) BVT.2733

(Fig. 2). Treatment with BVT.2733 lowered blood glucose and insulin concentrations. The blood glucose concentrations were 77±12% (n=17; p<0.01) of control and serum insulin concentrations were 67±26% (n=20; p<0.05) of control (Fig. 3A, B). Daily food intake, body weight, and serum AST, ALT and ALP were all unaffected by the BVT.2733 treatment.

Discussion

We describe that treatment with a selective inhibitor of 11β-HSD1, BVT.2733, results in lowering of blood glucose and serum insulin concentrations in a hyperglycaemic and hyperinsulinaemic mouse. BVT.2733 was administered with osmotic mini-pumps to obtain steady-state compound blood concentrations and enzyme inhibition. A pilot study was carried out to show that the 11β-HSD1 enzyme was inhibited in vivo. The results show that the inhibition closely reflects the serum and liver BVT.2733 concentrations. Thus, dilution of the tissue homogenate in the enzyme activity assay resulted in underestimation of the reversible inhibition in vivo.

It has been shown that *11β-HSD1* knock-out mice fed a high-fat diet have lower blood glucose concentrations than wild-type control mice [19, 21]. This difference however, was observed after the animals had been on the diet for 8 weeks. In our study, pharmacological inhibition of 11β-HSD1 in KKA^{y} mice lowered blood concentrations levels significantly (p<0.01) already within 7 days and was not paralleled by changes in daily food intake or body weight. Thus, both the previous gene disruption and the present BVT.2733 inhibition of 11β-HSD1 resulted in reduced blood glucose concentrations.

Administration of BVT.2733 reduced hepatic concentrations of mRNA encoding PEPCK and G6Pase – the rate-limiting enzymes for gluconeogenesis and the former only regulated on the mRNA level [6]. This is consistent with observations in *11β-HSD1* knock-out mice and supports the mechanistic rationale that glucocorticoids are permissive for excessive hepatic glucose production. In contrast to pharmacological inhibition, reductions of hepatic mRNA concentrations of PEPCK and G6Pase in *11β-HSD1* knock-out mice were observed only after fasting [19, 21]. Thus, it is likely, that our results underestimate the reduction in hepatic mRNA concentrations of PEPCK and G6Pase, and that pharmacological 11β-HSD1 inhibition possibly results in even larger reductions after a fasting period. Importantly, BVT.2733 administration for 7 days did not alter hepatic 11β-HSD1 mRNA concentration. The absence of any compensatory transcription of hepatic 11β-HSD1 mRNA after selective pharmacological inhibition of the enzyme is in agreement with a previous report on the use of the non-selective inhibitor glycyrrhizic acid in rats [23]. Thus, inhibition of 11β-HSD1 offers an effective pharmacological intervention that is likely to yield sustained reduction of glucocorticoid-inducible hepatic gluconeogenic enzymes.

The non-selective 11β-HSD1 inhibitor carbenoxolone has been used in a clinical study [18]. Then, glucose infusion rate was higher than control in a euglycemic hyperinsulinaemic clamp. Improved hepatic insulin sensitivity and decreased glucose production were also shown. However, the dose of the non-selective inhibitor (carbenoxolone) was limited by effects on the type 2 isoform of 11β-HSD in the kidney, inhibition of which yields hypertension [18].

BVT.2733 is a new, selective inhibitor for the 11β-HSD1 enzyme (with a K_i of 1 μmol/l) over the type 2 isoenzyme (no inhibition at 200 μmol/l). The affinity of BVT.2733 for other enzymes and receptors has not been investigated. However, six structurally related compounds have been subjected to a selectivity panel and were found to be devoid of any significant affinity ($p>0.05$ at 1 μmol/l) to a broad panel of enzymes and receptors, including the estrogen, glucocorticoid, progesterone, and androgen receptors (Cerep, Celle l'Evescault, France). Thus, BVT.2733 is from a class of selective 11β-HSD1 inhibitors. In support of the selectivity of BVT.2733, the results show that BVT.2733 did not alter common liver function marker enzymes such as AST, ALT, or ALP, indicating that the effects on hepatic glucose production can be ascribed to 11β-HSD1 inhibition.

In conclusion, administration of a selective 11β-HSD1 inhibitor lowered hepatic PEPCK and G6Pase mRNA, blood glucose and serum insulin concentrations in hyperglycaemic KKA^y mice. These results suggest that a selective inhibitor of human 11β-hydroxysteroid dehydrogenase type 1 can become a new approach for lowering blood glucose concentrations in Type II diabetes [24].

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Selective Inhibition of 11β-Hydroxysteroid Dehydrogenase Type 1 Improves Hepatic Insulin Sensitivity in Hyperglycemic Mice Strains

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11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) has been proposed as a new target for type 2 diabetes drugs. The aim of the present study was to assess the effects of inhibition of 11β-HSD1 on blood glucose levels, glucose tolerance, and insulin sensitivity in mouse models of type 2 diabetes. BVT.2733 is an isoform-selective inhibitor of mouse 11β-HSD1. Hyperglycemic and hyperinsulinemic *ob/ob*, *db/db*, KKA^y, and normal C57BL/6J mice were orally administered BVT.2733 (200 mg/kg·d, twice daily). In hyperglycemic, but not in normal mice, BVT.2733 lowered circulating glucose (to 50–88% of control) and insulin (52–65%) levels. In oral glucose tolerance tests in *ob/ob* and KKA^y mice, glucose concentrations were 65–75% of vehicle values after BVT.2733 treatment, and in KKA^y mice insulin concentrations were decreased (62–74%). Euglycemic, hyperinsulinemic clamps demonstrated decreased endogenous glucose production (21–61%). Analysis of hepatic mRNA in KKA^y mice showed reduced phosphoenolpyruvate carboxykinase mRNA (71%). A slight reduction in food intake was observed in *ob/ob* and KKA^y mice. Cholesterol, triglycerides, and free fatty acid levels were decreased to 81–86% in KKA^y mice after a 4-h fast. The results support previous suggestions that selective 11β-HSD1 inhibitors lower blood glucose levels and improve insulin sensitivity in different mouse models of type 2 diabetes. (*Endocrinology* 144: 4755–4762, 2003)

IT IS WELL established that glucocorticoids oppose the insulin effect in regulation of carbohydrate metabolism *in vivo*, including glucose uptake in peripheral tissues and hepatic glucose production (1). The liver plays a central role in maintaining glucose homeostasis, and patients with type 2 diabetes have increased hepatic glucose production, mainly due to increased gluconeogenesis (2). Glucocorticoids upregulate the genes encoding phosphoenolpyruvate carboxykinase (PEPCK), the rate-limiting enzyme for gluconeogenesis, and glucose-6-phosphatase (G6Pase), which regulates the outflow of glucose originating from either gluconeogenesis or glycogen degradation (3, 4). In man, iv cortisol infusion increases postabsorptive plasma glucose, insulin, and gluconeogenesis (5). The detrimental effects of chronic exposure to high circulating glucocorticoid levels are clearly illustrated by excess hepatic glucose production and type 2 diabetes observed in Cushing's syndrome (1).

Glucocorticoids exert their tissue effects by binding to the glucocorticoid and mineralocorticoid receptors, both members of the nuclear receptor superfamily of ligand-activated transcription factors. Administration of the glucocorticoid receptor antagonist RU 486 decreases blood glucose in *db/db* mice and in patients with Cushing's syndrome (6, 7). However, the specific effects on target tissues depend not only on receptor type and density, but also on the availability and metabolic conversion of glucocorticoid by intracellular enzymes (8). Two 11β-hydroxysteroid dehydrogenases (11β-HSD) catalyze the interconversion between active and inactive glucocorticoids (9). The enzyme 11β-HSD1 is widely expressed and yields increased local tissue concentration of active glucocorticoid by converting cortisone into cortisol in humans, and 11-dehydrocorticosterone into corticosterone in rodents. In contrast, the enzyme 11β-HSD2 catalyzes the opposite reaction, the inactivation of active glucocorticoid (10, 11).

The phenotype of mice having a targeted disruption of the 11β-HSD1 gene substantiates the role of 11β-HSD1 in the liver where it is highly expressed (12, 13). These mice have slightly lower plasma glucose levels on high-fat diet and show attenuated activation of key enzymes for hepatic gluconeogenesis after 24-h starvation combined with stress. Furthermore, 11β-HSD1 knockout mice exhibit improved glucose tolerance, which is displayed despite modestly elevated plasma corticosterone levels. This shows the pivotal role of 11β-HSD1 for enhancing intracellular glucocorticoid action *in vivo*.

BVT.2733 is a selective inhibitor of murine 11β-HSD1 shown to decrease blood glucose levels and gluconeogenic enzymes after 7-d continuous sc administration to hyperglycemic KKA^y mice (14). In the present study the effect of oral administration twice a day (b.i.d.) on glucose and insulin

Abbreviations: AUC, Area under the curve; b.i.d., *bis in diem*, twice a day; BVT.2733, 3-chloro-2-methyl-*N*-{4-[2-(4-methyl-1-piperazinyl)-2-oxoethyl]-1,3-thiazol-2-yl}benzenesulfonamide; EGP, endogenous glucose production; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GDR, glucose disposal rate; GIR, glucose infusion rate; G6Pase, glucose-6-phosphatase; 11β-HSD1, 11β-hydroxysteroid dehydrogenase type 1; OGTT, oral glucose tolerance test; PEPCK, phosphoenolpyruvate carboxykinase, p.o., *per os*, orally.

levels was tested in both KKA^y mice and two other established animal models of type 2 diabetes, the *ob/ob* and the *db/db* mice. Furthermore, the effect of BVT.2733 on endogenous glucose production, and insulin sensitivity was assessed in euglycemic, hyperinsulinemic clamp studies and glucose tolerance tests in conscious *ob/ob* and KKA^y mice.

Materials and Methods

Chemical substance

The compound 3-chloro-2-methyl-*N*-{4-[2-(4-methyl-1-piperazinyl)-2-oxoethyl]-1,3-thiazol-2-yl} benzenesulfonamide (BVT.2733) (15) was dissolved in 12% β-hydroxypropylcyclodextrin (Fluka, Buchs, Switzerland) and 0.3% sodium chloride (p.a. grade, Merck & Co., Darmstadt, Germany). Both the compound and vehicle were administered by oral gavage (p.o.) twice daily (b.i.d.) with 12-h intervals at 0700–0800 and 1900–2000 h for 3–4 d.

Animals

Male mice, C57BL/6J, C57BL/6JBom-*ob/ob* (*Lep^{ob}*; *ob/ob*; age 17–20 wk), and C57BL/KSBom-*db/db* (*Lepr^{db}*; *db/db*; age 12–17 wk), from Taconic Farms (Ry, Denmark) were given a normal diet (R34, Lactamin, Vadstena, Sweden) and water *ad libitum*. Male KK-A^y/Ta Jcl (KKA^y) mice (age, 8–14 wk; 10–12 wk old at the time of clamp) from Clea Japan (Tokyo, Japan) were given a high-fat diet (purified version of Sweetened Condensed Milk Diet, catalog no. D12266B; 32 kcal% fat, ~0.01% cholesterol; Research Diets, New Brunswick, NJ) and water *ad libitum*. The animals were kept one per cage at 22 ± 1 C, 50 ± 20% humidity, and a 12-h light, 12-h dark cycle with lights on at 0500–0630 h. They were grouped based on 4-h fasting blood glucose values, 4-h fasting blood glucose and plasma insulin (clamp studies), or body weight (normal, wild-type C57BL/6J mice). The procedures involving animals were in conformity with national and international laws for the care and use of laboratory animals and were approved by the local animal ethical committee.

Serum and plasma preparation

Serum was prepared from trunk blood samples that were kept 30 min at 4 C before centrifugation at 2000–3000 × *g* for 10–15 min and stored in tubes at −70 C until analysis.

Plasma was prepared from tail blood samples (10–20 μl) that were collected in heparinized hematocrit capillary tubes (Kebo-Lab, VWR, Solna, Sweden) on ice, centrifuged (24,000 × *g*, 10 min, 4 C), transferred to a microtiter 96-well plate, and stored at −20 C until analysis.

Glucose analysis

Blood glucose concentrations (Figs. 1–3 and Tables 2 and 3) were measured immediately using microcuvettes and test strips (Hemocue, Ängelholm, Sweden; or Accu-Chek, Roche, Basel, Switzerland). Both methods are based on glucose dehydrogenase conversion of glucose to gluconolactone. The measurable range is 0–22.2 mM for Hemocue and 0.6–33.3 mM for Accu-Chek. The reported within-run precision is SD ≤ 0.3 mM with coefficients of variation of 3.5%, 2.6%, 1.9%, 1.6%, and 2.2% at 4.3, 7.7, 12.5, 18.2, and 21.0 mM glucose, respectively (Hemocue), and with a coefficient of variation less than 4.0% at 9.2 mM and less than 2.9 SD at 3.3 mM glucose (Accu-Chek). When glucose concentrations exceeded the measurable range, samples were diluted with heparin (LEO, Lövens, Ballerup, Denmark; final concentration, 10 IU/ml in physiological saline) or physiological saline.

Serum glucose concentrations (Fig. 1) were measured with an UV method (Roche) using a Cobas Mira instrument. Glucose was oxidized to gluconolactone by glucose dehydrogenase in the presence of NAD^+ and mutarotase that accelerates the reaction. The formation of NADH was measured photometrically at 340 nm.

Insulin, cholesterol, triglyceride, and free fatty acid analysis

Serum insulin was analyzed with a rat insulin RIA (Linco Research, Inc., St. Louis, MO). In the oral glucose tolerance test (OGTT) and clamp experiments, plasma insulin was analyzed with a rat insulin ELISA (Mercodia, Uppsala, Sweden).

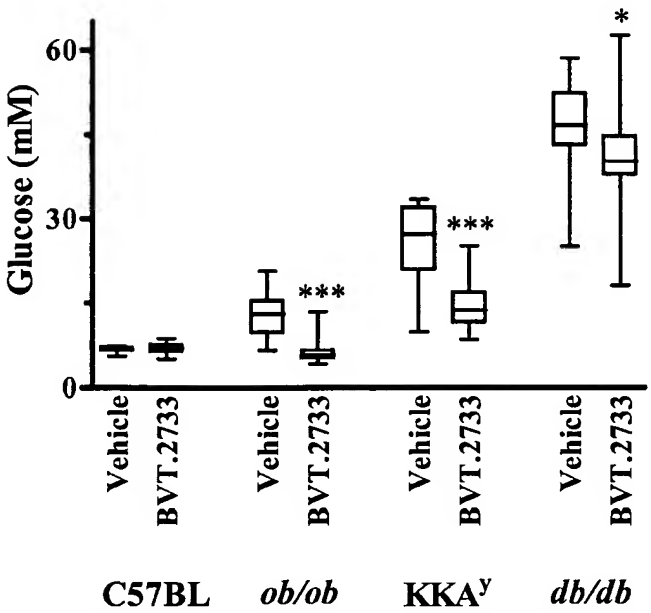

FIG. 1. Effect of BVT.2733 (200 mg/kg·d, p.o.) administration on blood glucose levels in normal C57BL/6J (n = 25 and 25) and spontaneously hyperglycemic *ob/ob* (n = 24 and 25), KKA^y (n = 16 and 16), and *db/db* (n = 22 and 22; serum glucose) mice measured 12 h after the last administration, which was once every 12 h for 4 consecutive days. Statistical difference from control: *, $P < 0.05$; ***, $P < 0.001$.

Serum cholesterol was analyzed with an enzymatic colorimetric method (MPR2, Roche). Serum triglycerides were analyzed with an enzymatic colorimetric method after elimination of free glycerol (Triglycerides/GB, Roche). Serum nonesterified, free fatty acids were determined with an enzymatic colorimetric method using oleic acid as a standard (NEFA C, Wako Chemicals, Neuss, Germany).

mRNA analysis

Real-time PCR was used to quantify mRNA levels (TaqMan, PE Applied Biosystems, Foster City, CA) of 11β-HSD1 (EC 1.1.1.146), PEPCK (EC 4.1.1.32), and G6Pase (EC 3.1.3.9) isolated from liver, mesenteric fat, and epididymal fat (14). Levels were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA and 18S rRNA. Plasmid containing cloned reference DNA was used to construct standard curves for 11β-HSD1and GAPDH levels (PE Applied Biosystems).

OGTT

All animals were administered BVT.2733 or vehicle b.i.d.; *ob/ob* mice BVT.2733 (200 mg/kg·d; for 4 d) and KKA^y mice BVT.2733 (400 mg/kg·d; for 3 d). D-Glucose (2 g/kg; 200 mg/ml stock solution, Fresenius Kabi, Uppsala, Sweden) was given p.o. after overnight fasting and 2 h after the last administration of vehicle or BVT.2733. Blood samples were taken immediately before the glucose load and after 15, 30, 60, and 120 min.

Euglycemic, hyperinsulinemic clamp

Surgery and drug treatment before clamp. A microrenathane implantation tubing catheter (Braintree Scientific, Inc., Braintree, MA) was inserted into the right jugular vein under isoflurane (Forene, Abbott, Chicago, IL) anesthesia at least 4 d before administration of drug or vehicle and at least 8 d before the clamp. The catheter was tunneled under the skin, extracted in the neck, and filled with glycerol containing heparin to prevent blood from clotting. BVT.2733 (200 mg/kg·d) or vehicle was administered for 3 d (KKA^y mice) and 4 d (*ob/ob* mice).

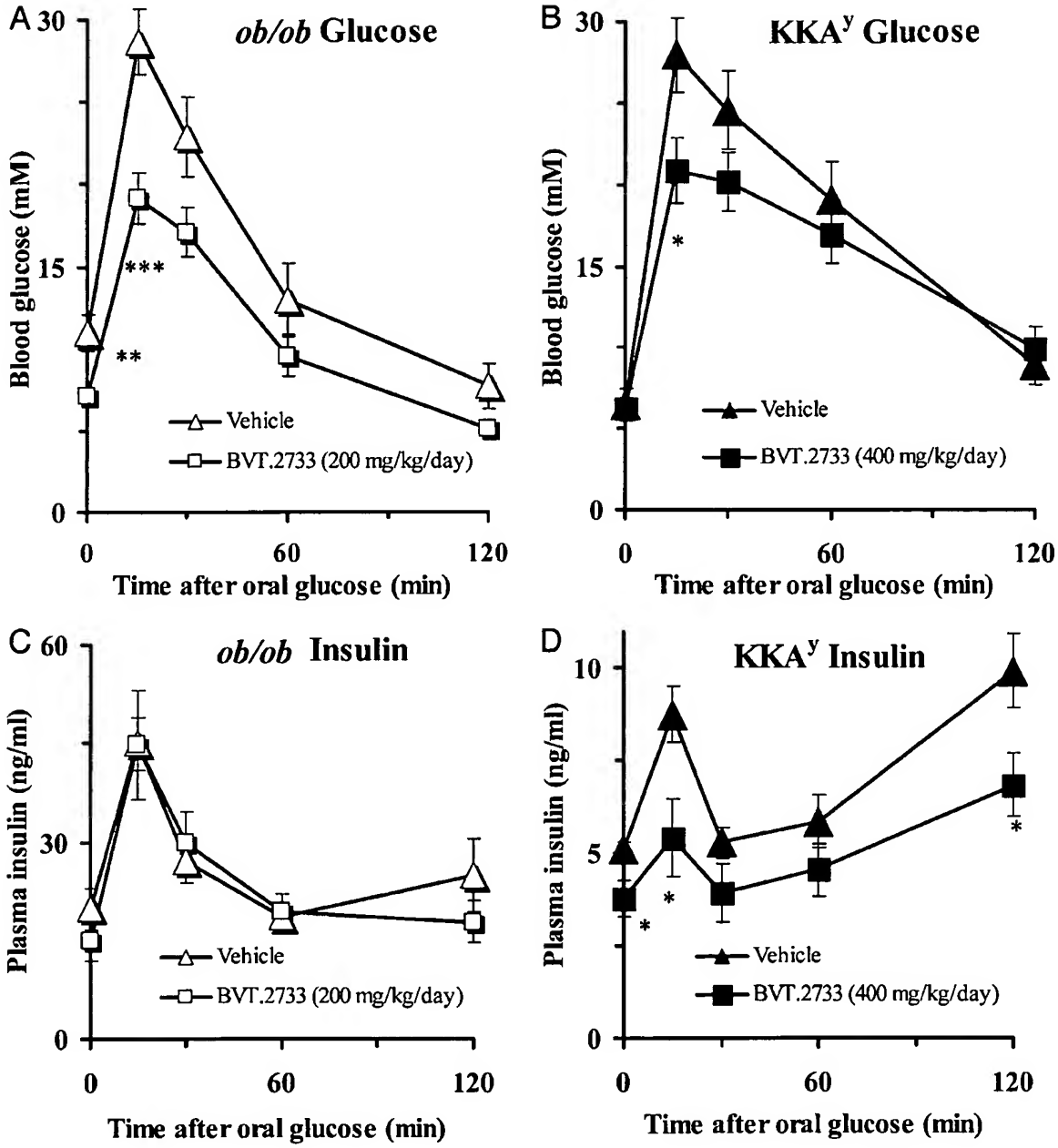

FIG. 2. OGTT. Spontaneously hyperglycemic *ob/ob* (A and C) and KKA^y (B and D) mice were treated with BVT.2733 (n = 12) or vehicle (n = 12), and after an overnight fast (14 h), D-glucose (2 g/kg, p.o.) was administered. A and B, Blood glucose concentrations. C and D, Plasma insulin concentrations. △ and ▲, Vehicle; ■ and □, BVT.2733; △ and □, *ob/ob* mice; ▲ and ■, KKA^y mice. Statistical difference from control: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Clamp protocol. The clamps were performed in conscious mice (16). After administration of the last BVT.2733 dose the animals were fasted for 5 h before start of the clamp procedure. The animals were connected to a liquid swivel via the venous catheter and acclimatized for 45 min.

3-D-[^{3}H]Glucose tracer (PerkinElmer, Boston, MA) dissolved in 0.9% saline was infused throughout the clamp to determine the glucose disposal rate (GDR). A priming bolus dose of 25 μCi/kg (925 kBq/kg) was given, followed by a continuous infusion of 2.5 μCi/kg·min (infusion rate, 50 μl/min·kg) by a microinjection pump (CMA/Microdialysis, Solna, Sweden). Blood samples (10 μl) were collected after 50, 60, and 70 min of D-[^{3}H]glucose infusion and were used to determine the basal GDR. At the same time points, basal blood glucose levels were measured, and at 70 min a 20-μl blood sample was taken for basal plasma insulin measurement.

Insulin infusion (Actrapid, Novo Nordisk, Bagsværd, Denmark) was started 70 min after initiation of D-[^{3}H]glucose infusion and continued for 90 min at a constant rate. A relatively low insulin concentration (12.5 mU/kg·min) was used to prevent complete suppression of endogenous glucose production (EGP) and therefore optimized to evaluate hepatic insulin sensitivity. Blood samples (10 μl) were taken after 70, 80, and 90 min of insulin infusion for determination of GDR. At 90 min, a 20-μl blood sample was taken for determination of circulating insulin levels during insulin infusion.

D-Glucose (300 mg/ml; Fresenius Kabi) was administered by an ad-

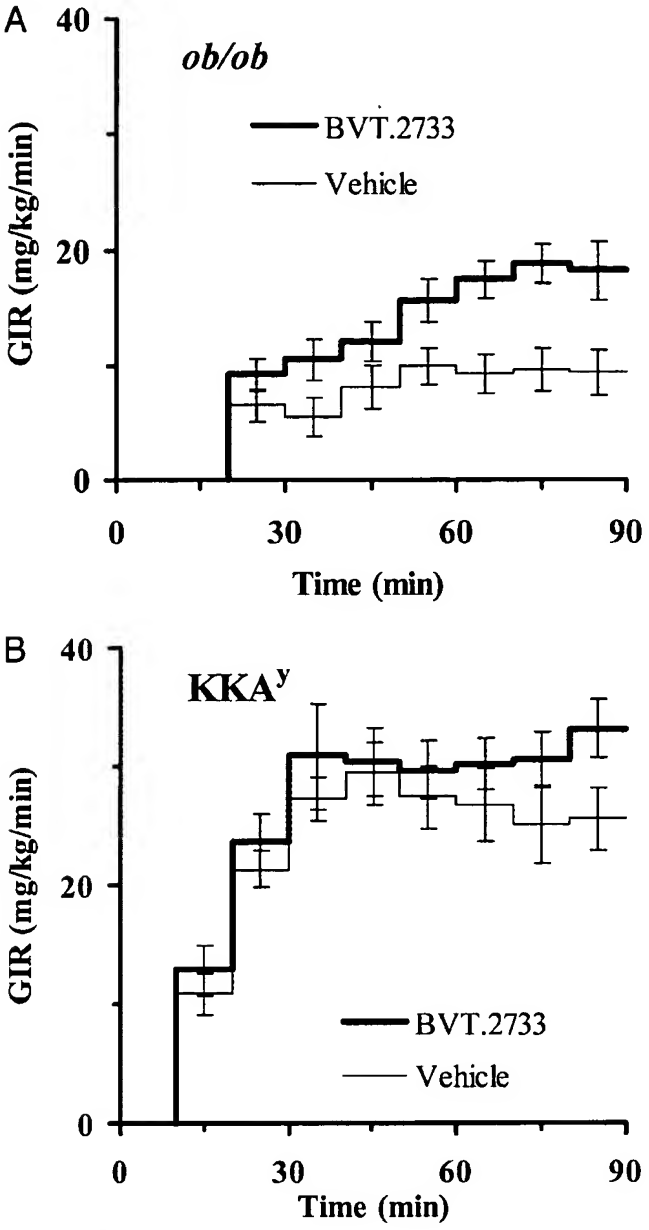

FIG. 3. GIR during euglycemic, hyperinsulinemic clamp in *ob/ob* mice (A; n = 10) and KKA^y mice (B; n = 8) after treatment with BVT.2733 (200 mg/kg·d, p.o.; *thick line*) or vehicle (*thin line*). For statistical differences at steady-state insulin infusion conditions (at 70–90 min), see Table 3.

justable infusion pump (model 100, KD Scientific, New Hope, PA) to maintain blood glucose at the same level as before insulin infusion. The glucose infusion rate (GIR) was guided by blood glucose concentration measurements every 10 min. Blood glucose was clamped at the individual basal blood glucose level.

Blood [^{3}H]D-glucose. Barium hydroxide (50 μl, 0.3 M) and zinc sulfate (50 μl, 0.3 M) were added to blood samples (10 μl) and mixed. After centrifugation, supernatant (25-μl) samples in duplicate were transferred to scintillation vials and allowed to evaporate to dryness overnight to eliminate [^{3}H]water. Next day, D-[^{3}H]glucose was dissolved in water (0.5 ml), scintillation fluid (3 ml Ultima Gold, Packard, Meriden, CT) was added, and radioactivity was measured in a liquid scintillation spectrometer (TriCarb, Packard, Meriden, CT).

Calculations. Blood glucose specific activity was calculated as the D-[^{3}H]glucose divided by the measured blood glucose levels. Steady-state conditions for blood glucose specific activity were achieved during basal and clamp periods in both *ob/ob* mice and KKA^y mice, as determined by linear regression analysis. The slopes were not significantly different from zero for all groups ($P > 0.05$; data not shown). GDR was calculated by dividing the rate of D-[^{3}H]glucose infusion by the average specific activity of blood glucose. During basal conditions, EGP is assumed to be equal to the GDR. Under steady-state insulin infusion conditions (at 70–90 min), it is assumed that EGP = GDR − GIR.

Results are expressed as the mean ± SEM, except in Figs. 1 and 4, where the box extends from the 25th to the 75th percentile, with a line at the median, and the error bars show the highest and lowest values (PRISM 3.0 software, GraphPad, San Diego, CA).

Statistics

Data from the OGTT and clamp were analyzed in two ways. OGTT area under the curve (AUC) data were calculated using a trapezoidal method with baseline adjustments. Clamp GIR AUC data were calculated using a rectangular method. Differences in AUC between compound- and vehicle-treated animals were analyzed using two-tailed, two-sample, equal variance (independent samples) *t* tests. Further, data were analyzed using repeated measurements ANOVA. In regression analyses, concerning analyses of calculated clamp EGP and GDR, the insulin-stimulated values were used as dependent variables, and basal values and treatment (0/1) were used as covariates. The nonparametric Mann-Whitney test was used when data significantly deviated from a normal distribution according to a Kolmogorov-Smirnov test. The glucose data were statistically assessed by independent sample *t* test. The mRNA data were statistically assessed by nonparametric Kruskal-Wallis test with Dunn's multiple comparisons test.

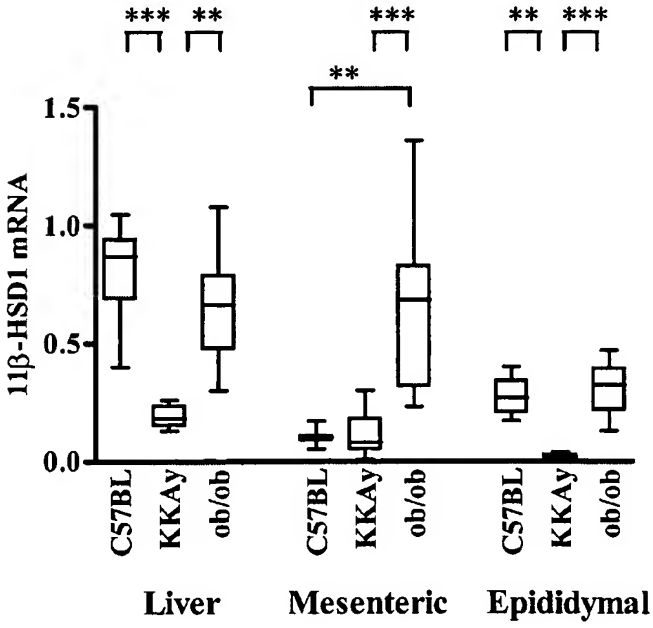

FIG. 4. Levels of 11β-HSD1 mRNA in C57BL/6J, KKA^y, and *ob/ob* mice given as relative units *vs.* GAPDH mRNA in liver, mesenteric fat, and epididymal fat (n = 9–10). Statistical difference is: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Results

Effect of the 11β-HSD1 inhibitor BVT.2733 on glucose, insulin, cholesterol, triglyceride, and free fatty acid concentrations; food intake; and body weight

BVT.2733 (200 mg/kg·d) administered by oral gavage (p.o.) b.i.d. for 4 d reduced circulating glucose concentrations in spontaneously hyperglycemic *ob/ob*, KKA^y, and *db/db* mice to 50%, 58%, and 88% of that vehicle-treated mice (Fig. 1). In contrast, BVT.2733 (200 mg/kg·d) did not alter the blood glucose concentration in normal C57BL/6J mice (Fig. 1).

Serum insulin concentrations were lowered by the BVT.2733 treatment in *ob/ob* and *db/db* mice and tended to be lowered in KKA^y mice, but were not altered in C57BL/6J mice (Table 1A).

Oral BVT.2733 (200 mg/kg·d) administration did not consistently alter serum cholesterol, triglyceride, and free fatty acid concentrations under *ad libitum*-fed conditions. However, cholesterol, triglycerides and free fatty acid levels were decreased in the KKA^y mouse after a 4-h fast (Table 1B). The fasting blood glucose was 69% of the control value.

Daily food intake was not affected in C57BL/6J and *db/db* mice by BVT.2733 (200 mg/kg·d). In *ob/ob* and KKA^y mice, food intake was decreased by 25–39% and 14–19% compared with vehicle. Body weight was not altered in C57BL/6J, KKA^y, and *db/db* mice, but was reduced by 5% in *ob/ob* mice compared with vehicle.

Effect of the 11β-HSD1 inhibitor BVT.2733 on hepatic mRNA concentrations

Treatment of KKA^y mice with BVT.2733 resulted in a dose-dependent reduction of PEPCK mRNA levels in the liver from 2.12 ± 0.16 for vehicle to 1.95 ± 0.10 and 1.51 ± 0.14 ($P < 0.01$; n = 8) after the administration of 100 and 200 mg/kg·d, respectively (relative units from normalization to GAPDH mRNA). The levels of 11β-HSD1 mRNA and G6Pase mRNA also tended to decrease. The level of 11β-HSD1 mRNA was 2.45 ± 0.44 for vehicle and 2.33 ± 0.25 and 1.75 ± 0.21 for BVT.2733 (100 and 200 mg/kg·d; n = 8). The level of G6Pase mRNA was 1.09 ± 0.19 for vehicle and 1.55 ± 0.19 and 0.78 ± 0.13 for BVT.2733 (100 and 200 mg/kg·d; n = 8). Similar results were obtained when data were normalized to 18S rRNA (not shown).

Effect of the 11β-HSD1 inhibitor BVT.2733 on OGTT

OGTTs were performed in *ob/ob* and KKA^y mice. In mice from both strains BVT.2733 treatment resulted in decreased blood glucose concentrations at early time points compared with vehicle treatment (Fig. 2). In the KKA^y mouse this was accompanied by lower insulin concentrations both before and after glucose administration, but in the *ob/ob* mice no differences in the effect on insulin levels were seen. ANOVAs using repeated measurements did not yield any significant differences between drug-treated and vehicle-treated mice. Also, the baseline adjusted AUCs (data not shown) for glucose and insulin in the KKA^y and *ob/ob* BVT.2733-treated and vehicle-treated mice did not differ significantly (independent sample t tests). In the KKA^y mice BVT.2733 (400 mg/kg·d) was used, because at 200 mg/kg·d no effect on the OGTT was observed in a pilot experiment (data not shown).

Effect of the 11β-HSD1 inhibitor BVT.2733 on insulin sensitivity

Blood glucose concentrations during steady-state insulin infusion in the euglycemic, hyperinsulinemic clamp were similar to basal levels in *ob/ob* mice and vehicle-treated KKA^y mice, but lower than basal levels in BVT.2733-treated KKA^y mice (Table 2). Plasma insulin was increased approximately 2- to 4-fold by the insulin (12.5 mU/kg·min) infusion (Table 2).

The GIR in BVT.2733-treated mice was higher than that in vehicle-treated mice at all time points in both *ob/ob* and KKA^y mice (Fig. 3 and Table 3). In *ob/ob* mice the corresponding AUCs between BVT.2733- and vehicle-treated mice were also significantly different ($P < 0.01$; data not shown).

TABLE 1. Effect of BVT.2733 administration on serum insulin, cholesterol, triglyceride, and free fatty acid concentrations

Mouse strain	Insulin (ng/ml)	Cholesterol (mM)	Triglycerides (mM)	Free fatty acids (mM)	n
A. Effect of 4-d BVT.2733 (200 mg/kg·d) administration on *ad libitum*-fed serum concentrations 12 h after dose					
C57BL/6J					
Vehicle	1.0 ± 0.078	2.9 ± 0.064	1.1 ± 0.043	0.65 ± 0.032	24
BVT.2733	1.2 ± 0.15	2.9 ± 0.081	1.0 ± 0.045	0.63 ± 0.035	25
ob/ob					
Vehicle	96.3 ± 12.3	6.0 ± 0.15	1.1 ± 0.082	0.67 ± 0.031	24
BVT.2733	59.7 ± 11.6[a]	6.5 ± 0.17[a]	0.91 ± 0.038	0.47 ± 0.025[b]	25
KKA^y					
Vehicle	32.8 ± 5.2	4.1 ± 0.21	3.1 ± 0.32	1.5 ± 0.10	15
BVT.2733	21.4 ± 2.4	3.3 ± 0.14	2.2 ± 0.15	1.1 ± 0.058	16
db/db					
Vehicle	2.7 ± 0.50	2.6 ± 0.13	1.2 ± 0.10	0.81 ± 0.046	22
BVT.2733	1.4 ± 0.32[a]	2.7 ± 0.15	1.2 ± 0.12	1.1 ± 0.083[a]	22
B. Effect of 3-d BVT.2733 (200 mg/kg·d) administration on 4-h fasting serum concentrations 4 h after dose					
KKA^y					
Vehicle	11.8 ± 1.1	4.2 ± 0.11	2.4 ± 0.16	0.90 ± 0.042	30
BVT.2733	10.6 ± 0.96	3.6 ± 0.14[b]	2.0 ± 0.10[a]	0.73 ± 0.029[c]	29

The same animals were used as those in Fig. 1.
[a] $P < 0.05$.
[b] $P < 0.001$.
[c] $P < 0.01$.

TABLE 2. Blood glucose and plasma insulin concentrations at steady state during euglycemic, hyperinsulinemic clamp for vehicle, and BVT.2733 (200 mg/kg·d)

Mouse strain	Blood glucose (mM)		Plasma insulin (ng/ml)	
	Basal	Insulin infusion	Basal	Insulin infusion
ob/ob				
Vehicle (n = 10)	7.3 ± 0.83	7.4 ± 0.79	24.3 ± 5.8	66.1 ± 13.2[a]
BVT.2733 (n = 10)	5.4 ± 0.39	5.4 ± 0.47[b]	20.2 ± 4.2	38.5 ± 10.5[c]
KKA^y				
Vehicle (n = 8)	14.0 ± 1.9	13.7 ± 1.8	8.4 ± 1.8	25.9 ± 1.8[a]
BVT.2733 (n = 8)	10.9 ± 2.1	9.7 ± 1.9[d]	6.7 ± 2.0	25.2 ± 3.0[a]

[a] $P < 0.001$ *vs.* basal.
[b] $P < 0.05$ *vs.* vehicle.
[c] $P < 0.05$ *vs.* basal.
[d] $P < 0.01$ *vs.* basal.

TABLE 3. Effect of BVT.2733 (200 mg/kg·d) on glucose disposal rate (GDR), glucose infusion rate (GIR), and endogenous glucose production (EGP)

Mouse strain	GDR (mg/kg·min)		GIR (mg/kg·min)	EGP (mg/kg·min)	
	Basal	Insulin infusion	Insulin infusion	Basal	Insulin infusion
ob/ob					
Vehicle (n = 10)	27.8 ± 4.9	28.7 ± 1.7	9.4 ± 1.7	27.8 ± 4.9	19.4 ± 2.3
BVT.2733 (n = 10)	25.5 ± 4.0	29.5 ± 1.2	17.7 ± 1.7[a]	25.5 ± 4.0	11.8 ± 1.6[b,c]
KKA^y					
Vehicle (n = 8)	35.1 ± 4.9	44.3 ± 5.2[d]	25.7 ± 2.9	35.1 ± 4.9	18.5 ± 3.6[b]
BVT.2733 (n = 8)	26.1 ± 2.8	35.1 ± 2.4[b]	31.3 ± 2.3	26.1 ± 2.8	3.8 ± 1.0[e]

The GDR, GIR, and EGP values were calculated from samples obtained after 70–90 min of insulin infusion (*cf.* Fig. 3).
[a] $P < 0.01$ *vs.* vehicle.
[b] $P < 0.01$ *vs.* basal.
[c] $P < 0.05$ *vs.* vehicle.
[d] $P < 0.05$ *vs.* basal.
[e] $P < 0.001$ *vs.* basal. $P < 0.001$ *vs.* vehicle.

The GDR was increased during insulin infusion in both vehicle- and BVT.2733-treated KKA^y mice, but was not different between vehicle- and BVT.2733-treated *ob/ob* mice (Table 3). Under basal conditions no difference was observed between vehicle- and BVT.2733-treated groups in any of the strains.

EGP under basal conditions tended to be lower in BVT.2733-treated than in vehicle-treated *ob/ob* and KKA^y mice (Table 3). During insulin infusion, however, EGP was more suppressed in both *ob/ob* and KKA^y mice treated with BVT.2733 compared with vehicle-treated mice (Table 3).

Comparison of 11β-HSD1 mRNA levels in liver and two different fat depots

The two hyperglycemic models in which the effects of 11β-HSD1 inhibition were studied most extensively were further characterized in terms of 11β-HSD1 mRNA levels. The levels of 11β-HSD1 mRNA (normalized to GAPDH mRNA) in the liver, mesenteric fat, and epididymal fat were determined in C57BL/6J, KKA^y, and *ob/ob* mice (Fig. 4). In liver and epididymal fat, the levels were higher in C57BL/6J and *ob/ob* than in KKA^y mice. In mesenteric fat the levels in *ob/ob* mice were higher than those in C57BL/6J and KKA^y mice.

Discussion

Excess tissue glucocorticoid action may underlie several characteristics of type 2 diabetes and the metabolic syndrome. Selective inhibition of 11β-HSD1 provides the means to block local activation of glucocorticoids. The selective 11β-HSD1 inhibitor BVT.2733 has recently been shown to decrease blood glucose, serum insulin, and hepatic PEPCK mRNA levels in hyperglycemic and hyperinsulinemic KKA^y mice when administered continuously by sc osmotic minipumps (14). The present study aimed to investigate the physiological mechanisms behind the effects of 11β-HSD1 inhibition on glucose homeostasis and insulin sensitivity and to explore the effects of oral BVT.2733 administration in three different hyperglycemic and hyperinsulinemic mouse strains, *ob/ob*, *db/db*, and KKA^y, as well as in normal C57BL/6J mice.

The present results show that oral BVT.2733 administration decreased circulating glucose levels in *ob/ob*, *db/db*, and KKA^y mice, with a parallel decrease in insulin levels. Analysis of hepatic mRNA in the KKA^y mouse showed reduced levels of PEPCK mRNA. Thus, the results using oral administration confirm and extend the results obtained after osmotic minipump administration in the KKA^y mouse (14). In the current study a larger effect of 11β-HSD1 inhibition on blood glucose was seen in *ob/ob* and KKA^y mice compared with *db/db* and normal mice. The *ob/ob* mice displayed the same concentration of 11β-HSD1 mRNA in the liver as normal mice, whereas KKA^y mice had only 25%. Furthermore, it has been reported that both the 11β-HSD1 activity and mRNA levels are elevated in livers of *db/db* mice (17). Thus, the magnitude of the reduction of hepatic glucose production and blood glucose levels depends not only on the level of the target enzyme, but on other factors as well. In accordance, no

blood glucose decrease was observed in normal mice despite high levels of 11β-HSD1 expression in the liver. The lack of hypoglycemic effect in normal mice is in agreement with previous data showing that the nonspecific 11β-HSD1 inhibitor carbenoxolone does not yield decreased blood glucose in healthy human volunteers (18, 19). Furthermore, 11β-HSD1 knockout mice display normal fasting glucose levels (12, 20). Thus, inhibition and lack of the enzyme yield similar results. Taken together, the data support a fine-tuning effect of 11β-HSD1 on blood glucose levels, where inhibition leads to a decrease in excessive, but not basal, levels.

After a 4-h fast, blood glucose as well as serum cholesterol, triglycerides, and free fatty acids were decreased in KKA^y mice given BVT.2733. However, samples taken under *ad libitum*-fed conditions did not consistently alter serum cholesterol, triglyceride, or free fatty acid concentrations. As the fasting samples were obtained 4 h after administration, whereas samples from *ad libitum*-fed animals were collected 12 h post treatment, higher levels of inhibitor may have contributed to the decreases in lipids. Thus, lipid lowering might have a beneficial effect on hepatic insulin sensitivity and contribute to the reduced blood glucose levels. A lipid-lowering effect from 11β-HSD1 inhibition was expected based on the lipolysis-inducing properties of glucocorticoids (19, 21). Similarly, selective overexpression of 11β-HSD1 in white adipose tissue leads to increased adipose levels of corticosterone, lipoprotein lipase mRNA, and circulating free fatty acids (22). These transgenic mice develop visceral obesity similar to the human metabolic syndrome, supporting the hypothesis that 11β-HSD1 inhibition might prevent or reduce omental obesity in human metabolic syndrome and type 2 diabetes. Clinical data suggest that overexpression of 11β-HSD1 might contribute to development of the metabolic syndrome, because increased obesity correlates with higher expression of the enzyme (23, 24). However, in humans only sc adipose tissue has been investigated, not the more metabolically active and glucocorticoid receptor-rich visceral adipose tissue, where fat accumulation has been shown to correlate with increased morbidity and mortality (25).

Hyperglycemia and hyperphagia appear to depend on glucocorticoids in both man (23) and the commonly used models for type 2 diabetes *ob/ob*, *db/db*, and KKA^y mice and *fa/fa* Zucker rats (26–28). In *ob/ob* and *db/db* mice, adrenalectomy slows and cortisone treatment increases weight gain (29). Likewise, the glucocorticoid receptor antagonist RU 486 prevents development of hyperphagia, obesity, and fat deposition in *fa/fa* Zucker rats with no effect in lean rats (30, 31). Moreover, mice exhibiting enhanced glucocorticoid activity due to selective overexpression of 11β-HSD1 in white adipose tissue are both hyperglycemic and hyperphagic (22). In accordance, the present study shows that 11β-HSD1 inhibition yielded slightly decreased food intake in *ob/ob* and KKA^y mice. This is expected to contribute to reduced blood glucose levels. However, in KKA^y mice the reduction in food intake was smaller than that in blood glucose, and in *db/db* mice glucose levels were reduced, with no concomitant effect on food intake. Thus, a beneficial reduction in food intake may contribute to the blood glucose-lowering effect of 11β-HSD1 inhibition, although to a different degree in different models of type 2 diabetes.

Glucocorticoids contribute to increased hepatic glucose output in diabetes (4) and counteract the actions of insulin (1). The enzyme 11β-HSD1 enhances the effect by generating active glucocorticoid in target tissues (9). We have previously shown that BVT.2733 inhibits 11β-HSD1 in the liver and reduces hepatic levels of mRNA corresponding to key gluconeogenic enzymes (14). The present results support the hypothesis that selective 11β-HSD1 inhibition yields enhanced insulin sensitivity and agrees with the clinical finding of improved whole body insulin sensitivity using the nonselective 11β-HSD1 inhibitor carbenoxolone (18). The euglycemic, hyperinsulinemic clamp data from the present study show improvement in hepatic insulin sensitivity in both *ob/ob* and KKA^y mice; in both models, EGP was more suppressed during insulin infusion in mice treated with BVT.2733 than in vehicle-treated control animals. Also under basal conditions EGP tended to be lower in BVT.2733-treated *ob/ob* and KKA^y mice. The GIR was higher in *ob/ob* mice treated with the selective 11β-HSD1 inhibitor compared with the vehicle-treated group, and in KKA^y mice treatment with BVT.2733 tended to increase the GIR. In the latter case the GIR may have been underestimated, as the animals did not fully maintain basal glucose levels at steady-state insulin infusion conditions.

The GDR increased during insulin infusion in both vehicle- and BVT.2733-treated KKA^y mice, whereas no effect of BVT.2733 administration was detected. However, a tendency toward increased GDR was seen in *ob/ob* mice after BVT.2733 treatment, which might be a reflection of higher levels of the target 11β-HSD1 in adipose tissue compared with KKA^y mice. Furthermore, preliminary observations of 11β-HSD1 inhibition by BVT.2733 in adipose tissue suggest that peripheral effects are likely (data not shown). However, the primary objective of the present study was to evaluate the effect on hepatic insulin sensitivity. Therefore, mild hyperinsulinemia was employed in the clamp studies to avoid complete suppression of hepatic glucose production. A more pronounced hyperinsulinemia would have been used to reveal effects on peripheral insulin sensitivity. Such levels were employed in a pilot study where insulin was infused at 25 mU/kg·min in KKA^y mice treated with BVT.2733 (400 mg/kg·d) for 3 d. In that study enhanced hepatic insulin sensitivity (EGP) as well as a trend toward increased GDR were seen (data not shown).

In *ob/ob* and KKA^y mice BVT.2733 treatment resulted in reduced glucose levels that were accompanied by reduced or unchanged insulin levels during the OGTT. This suggests improved whole body glucose tolerance and increased insulin sensitivity. The results are in accordance with data on 11β-HSD1 gene knockout mice exhibiting improved glucose tolerance (13) and with impaired glucose tolerance in transgenic mice having increased 11β-HSD1 activity in white adipose tissue (22). A higher dose of BVT.2733 (400 mg/kg·d) was required for effects on glucose tolerance in the KKA^y model, in which a larger effect on insulin and a lesser effect on glucose levels were observed. Thus, there are qualitative differences between the two models, which may reflect different levels of target enzyme not only in liver and adipose tissue, but also in skeletal muscle and pancreas.

In conclusion, oral administration of a selective 11β-HSD1

inhibitor resulted in lowered circulating glucose and insulin levels in three separate mouse models of type 2 diabetes, but not in normal mice, yielding further support to the suggestion that a selective inhibitor of human 11β-HSD1 may lower blood glucose levels in subjects with type 2 diabetes (14) without causing hyperglycemia. Decreased EGP suggests enhanced insulin sensitivity. In addition, a reduction in food intake was seen in some of the models, suggesting a beneficial reduction in weight gain over time.

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Part of the results has been presented in preliminary form (32, 33).

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P45

Transcriptional interference by novel human PPARgamma mutants associated with lipodystrophic insulin resistance

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The nuclear receptor PPARgamma is important for biological processes including adipogenesis and glucose homeostasis. In subjects with severe insulin resistance, we have previously reported two types of human PPARgamma gene defect: heterozygous, missense mutations (P467L, V290M) in the ligand binding domain (LBD) which inhibit wild type (WT) receptor action in a dominant negative manner by recruitment of transcriptional corepressors; or double heterozygosity for a frameshift/premature stop (FSX mutation in the DNA binding domain (DBD) of PPARgamma leading to loss of receptor function, together with a defect in a second gene (PPP1R3) involved in skeletal muscle glycogen metabolism.

Here, we describe three novel heterozygous mutations in the DNA (C114R, C131R) or ligand (R357X) binding domains of PPARgamma in unrelated subjects with the characteristic clinical phenotype including a stereotyped pattern of partial lipodystrophy, insulin resistance, dyslipidaemia and hypertension. Consonant with their location, the novel PPARgamma mutations disrupt DNA binding (C114R, C131Y) or dimerisation (R357X) with retinoid X receptor and are transcriptionally inactive like the FSX mutation. However, in contrast to the FSX mutant, the C114R, C131Y and R357X mutant proteins retain the ability to translocate to the nucleus. Furthermore, the C114R, C131Y and R357X mutants inhibit WT receptor action when coexpressed, whereas the FSX mutant lacks dominant negative activity. Induction of a PPARgamma target gene (aP2) in response to receptor agonist was markedly attenuated in primary monocyte-derived dendritic cells from subjects harbouring the C114R, C131Y and R357X mutations whereas cells with the FSX mutation responded fully, providing *in vivo* evidence for the differing dominant negative properties of mutant receptors. Our observations suggest that dominant negative inhibition by the new PPARgamma mutants occurs via a novel mechanism, with competition for coactivators interfering with transcriptional activation by wild type receptor.

P46

Single nucleotide polymorphisms within oxidative stress genes affect arterial compliance: gene function relationships

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Background and Hypothesis:- A common *NOS3* Single nucleotide polymorphisms (SNP) (894 G/T) which encodes a Glu298Asp amino acid substitution in endothelial NO synthase has been associated with cardiovascular disorders in which NO bioactivity is impaired. The gene coding for p22phox, a critical component of the NADH/NAD(P)H oxidase enzyme system, a major source of vascular SO, is *CYBA*. Among the allelic polymorphisms reported in *CYBA* is C242T, associated with progression of coronary atherosclerosis. Consistent characteristic changes in the pressure pulse waveshape have been associated with ageing, risk factors for cardiovascular disease and impaired NO bioactivity. Therefore pulse waveform analysis (PWA) can be utilized to quantify arterial compliance as well as dynamic changes in NO bioactivity. We hypothesise that the two SNPs within oxidative stress genes influence vascular compliance in patients with CAD.

Methods:- We recruited 100 patients with angiographically documented coronary artery disease. Genotypes were determined with polymerase chain reaction and restriction digestion. Radial artery pressure waveforms were recorded using a calibrated tonometer. Windkessel based diastolic pressure decay analysis was then used to generate large (C1) and small (C2) artery compliance values. Differences between genotype groups were analysed using unequal variance unpaired Student's t tests.

Results:- The distribution of the genotypes in either gene did not differ significantly from that expected under Hardy Weinberg equilibrium. Homozygosity for a common *NOS3* polymorphism (894 G/T) was associated with decreased small artery compliance ($p = 0.006$) but not with large artery compliance or blood pressure. In contrast the *CYBA* 242T allele was associated with decreased large artery compliance ($p=0.042$) and increased systolic ($p=0.005$) and pulse pressure ($p=0.001$).

Conclusion:- We confirmed our hypothesis that SNPs in the *NOS3* and *CYBA* genes contribute to vascular compliance thus implicating the PWA as a cardiovascular intermediate phenotype.

P47

A novel non-steroidal inhibitor of 11beta-hydroxysteroid dehydrogenase type 1 improves features of metabolic syndrome in murine disease models

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Chronic exposure to elevated circulating glucocorticoids can lead to metabolic changes, which resemble those observed in Metabolic Syndrome. Features of the Metabolic Syndrome can be reversed by lowering systemic glucocorticoid levels or by treatment with a glucocorticoid receptor antagonist. At the intracellular level, exposure to glucocorticoids is regulated by two distinct 11beta-hydroxysteroid dehydrogenases, 11beta-HSD type 1 and type 2. 11beta-HSD1 predominantly acts as a reductase, converting inactive cortisone (11-dehydrocorticosterone in rodents) to active cortisol (corticosterone), whereas 11beta-HSD2 is a dehydrogenase responsible for glucocorticoid inactivation. Studies with transgenic mice have elucidated the importance of intracellular metabolism of glucocorticoids by 11beta-HSD1 for the development of Metabolic Syndrome.

High-throughput screening identified a novel potent and selective adamantyl triazole enzyme inhibitor of 11beta-HSD1. The ability of the compound to inhibit enzyme activity in mice was confirmed by pharmacodynamic assessment after oral dosing. In diabetic mice maintained on a high-fat diet and treated with a single moderate dose of streptozotocin, chronic oral administration (30 mpk b.i.d., 9 days) of the 11beta-HSD1 inhibitor resulted in lowered serum triglycerides (16-40%), lowered fasting glucose (77-80%) and decreased glucose excursions in glucose tolerance tests. Treatment (20 mpk b.i.d., 11 days) of diet-induced obese mice with the inhibitor lowered insulin (40-71%) and fasting glucose (10-28%) and reduced body weight (5-12%).

In summary, pharmacological inhibition of 11beta-HSD1 in mice leads to profound improvement of several key features of the Metabolic Syndrome.

P48

Mechano-receptors and cell-to-cell communication in the human collecting duct: functional involvement of TRPV4 and connexin-43

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Background: Transient Receptor Potential (TRP) channels are expressed in many epithelial cells and respond to mechanical or osmotic stress by initiating a number of calcium-dependent mechanisms. The human serum and glucocorticoid regulated kinase (hSGK) is one such calcium-dependent protein found in the kidney. Stimulated by cell shrinkage, this volume-regulated kinase stimulates sodium transport. Aberrant absorption of sodium has been implicated in the development of hypertension associated with renal disease and diabetic nephropathy. Osmotic-induced changes in cell volume can have dramatic repercussions for connexin-mediated cell-to-cell contact and may further exacerbate sodium-induced hypertension.

Objectives: In the present study we have utilised a novel cell line of the human collecting duct (HCD), as a model system to examine expression and localisation of these key signal recognition response elements and have investigated the extent of direct cell-to-cell communication.

Results: Mechanical stimulation of fura-2-loaded HCD cells evoked a transient increase in cytosolic calcium (5 separate experiments). The heptanol-sensitive calcium signal propagated rapidly (30plus/minus9.4microns/sec) across adjacent cells within a defined cell cluster (10-15 cells/cluster). Microinjected Lucifer yellow, permeated within cell clusters and confirmed a high degree of direct cell-coupling between HCD cells. Expression of TRPV4, connexin-43 (Cx-43) and hSGK mRNA was confirmed by RT-PCR. Western blotting of HCD cells identified Cx-43 and hSGK protein expression and immunocytochemistry localised hSGK mainly to the nucleus and Cx-43 to the cytosol and plasma membrane.

Conclusions: These data suggest that the TRPV4 mechanoreceptors mediate touch-evoked changes in intracellular calcium in HCD-cells. The evoked calcium-signal propagates across HCD cell clusters via gap-junctions. These touch-evoked calcium-signals, that mimic osmotically-driven changes in cytosolic calcium, may have important implications for hSGK-mediated sodium re-absorption and the development of hypertension.

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GENES AND TARGETS

1-OR

Identification of Adipocyte-Specific Genes in Obesity from Various Hmga2 Genotypes

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HMGene has developed the Adiposense ™ technology to identify adipocyte specific obesity targets from the various Hmga2 animal models of adipocyte differentiation. The Hmga2 null mouse adipose tissue is predominantly composed of preadipocytes, and the Hmga2 null mouse that is leptin deficient has adipose tissue that is predominantly composed of adipocytes. The RNA isolated from the adipose tissue of the Hmga2 null and the leptin deficient-Hmga2 null mouse was subjected to differential gene expression analysis. Twenty-five genes that were highly expressed in the latter tissue were identified. Eleven are ESTs and the remaining 14 are known genes. Six out of the fourteen known genes were previously identified adipocyte specific genes and include leptin, AdipoQ, resistin, PEPCK, S3-12, and FSP27. Independent verification of the remaining eight genes demonstrated that these genes are expressed 10 to 100-fold higher in the adipocyte as compared to the stromal-vascular fraction.

Two of the genes obtained by Adiposense ™ were evaluated as obesity targets. The first gene, HM-21, encodes a secreted protein. Transgenic mice that expressed the secreted protein were generated. These mice have substantially less adipose tissue as compared to their wildtype littermates. The second gene encodes a receptor. Null mice for this receptor have a reduced amount of adipose tissue as compared to their wildtype and heterozygous littermates. Thus, the modulation of both the genes obtained through Adiposense ™ has resulted in the development of mice with reduced fat pads.

The in vivo manipulation of the Hmga2 gene, which is involved in adipocyte proliferation and differentiation, has led to the identification of novel adipocyte specific genes that are putative obesity targets.

2-OR

Discovery of a Novel Class of Nonsteroidal, Potent and Selective 11ß-Hydroxysteroid Dehydrogenase Inhibitors

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11b-Hydroxysteroid dehydrogenase type 1 catalyses the inter-conversion of inactive cortisone into bioactive cortisol principally in liver and adipose tissue. Visceral adiposity is a high-risk factor in developing the clinical features of the metabolic syndrome which includes insulin resistance, diabetes and hyperlipidemia. In human, visceral obesity has been associated with locally increased cortisol levels and higher HSD1-activity. Transgenic mice over-expressing HSD1 specifically in adipose tissue, develop visceral adiposity, insulin resistance and diabetes, mimicking the clinical traits observed in human. HSD1-null mice exhibit improved lipid profile, hepatic insulin sensitivity and glucose tolerance, suggesting that HSD1 inhibition could be a therapeutic target for developing new drugs to overcome the morbid conditions associated with the metabolic syndrome. In support for this hypothesis, carbenoxolone, a nonselective HSD1 inhibitor, has been shown to increase hepatic insulin sensitivity in type 2 diabetic patients.

From High Throughput Screening (HTS) and further optimisation, we have identified a novel class of highly potent and selective HSD1-inhibitors. Representatives of this class of compounds inhibit HSD1-activity in 3T3-L1 cells (IC50: 4 nM), in primary human adipocytes (IC50: 0.3 nM), and in primary human hepatocytes (IC50: 13 nM). Compounds from this class are more than 100 fold selective over HSD2 activity measured in HepG2 cells (IC50: 1.2mM). They inhibit the in vitro differentiation of 3T3-L1 cells with an IC50 value < 100 nM. Oral administration of some of these compounds inhibit the acute conversion of cortisone into cortisol with ED50 values < 1 mpk. PO administration of one of these compounds for 2-4 weeks reduces body fat mass and fasting glucose in KKAy-mice, DIO and db/db mice by 20-25 % without affecting food intake.

3-OR

Evidence Against an Endocrine Role for Resistin, TNF-alpha and IL-6 in Upper Body Obesity

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Plasma adipokine (adiponectin, resistin, TNF-α, IL-6) concentrations are thought to be related to fatness/insulin resistance and are hypothesized to impact insulin resistance via endocrine mechanisms. We assessed the relationship amongst plasma adipokine concentrations and with insulin sensitivity in upper body obesity (UBO) before and after either diet/exercise (Diet/ex) or pioglitazone (PIO) treatment to improve insulin action. Plasma adipokine concentrations, insulin sensitivity (IVGTT) and body composition (abdominal CT and DEXA) were assessed in 39 UBO insulin resistant, non-diabetic men and women (BMI 32.8 ± 0.4 kg/m^2) before and after 19 weeks of Diet/ex or 30 mg/day PIO. Si ranged from 0.71 to 19.9 (mU/l)$^{-1}$• min^{-1}. With Diet/ex volunteers lost 9.3 ± 0.9 kg of body fat ($p< 0.001$), improved Si (5.3 ± 1.0 vs. 10.3 ± 1.9) and fasting glucose (96 ± 2 vs. 92 ± 2 mg/dl, both $p<0.05$). With PIO total body fat increased (1.3± 0.4 kg, $p<0.01$); Si improved (4.2 ± 0.6 vs. 6.6 ± 1.1) and fasting glucose declined (97 ± 2 vs. 92 ± 2 mg/dl). Adipokine concentrations were not correlated with each other, except pre-intervention TNF-α with IL-6 ($r = 0.43$, $p<0.05$). IL-6 inversely correlated with resistin. Only adiponectin correlated with baseline Si ($r=0.33$), insulin ($r= -0.38$) and C-peptide ($r= -0.36$; all $p<0.05$). Insulin sensitivity at baseline and after the interventions correlated inversely with resistin. Adiponectin increased more after PIO (4770 ± 487 vs. 8351 ± 694 ng/ml, $p<0.001$) than after diet/ex (4704 ± 367 to 5426 ± 325 ng/ml, $p<0.01$), whereas TNF-α, IL-6 and resistin did not change. Correlations between improved insulin sensitivity and changes in resistin and IL-6 that reached statistical significance were in the opposite direction from predicted. Thus, in non-diabetic UBO adults, plasma adipokine concentrations are not consistently related with each other or with insulin sensitivity. Only adiponectin displayed the expected relationship with insulin sensitivity . These findings do not support an endocrine role for resistin, TNF-α and IL-6 in insulin resistance.

4-OR

Small Molecule Inhibitors of Stearoyl-CoA Desaturase-1 Reduce Body and Adipose Weight Gain in Rat Models of Obesity

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Stearoyl-CoA Desaturase (SCD1) catalyzes the desaturation of the saturated fatty acids stearic acid (18:0) and palmitic acid (16:0) to produce oleic acid (18:1n9) and palmitoleic acid (16:1n7). Mouse knockout studies show that reduction of SCD1 activity is associated with beneficial changes in metabolism, including reduced body and adipose weight gain, lower triglycerides and improved insulin sensitivity. Human studies demonstrated that increased SCD1 activity, as assessed by fatty acid content of plasma or muscle tissue, is associated with high BMI, insulin resistance, high triglycerides and low HDL. Together these studies indicate SCD1 as an ideal target for therapeutic intervention for the treatment of obesity and metabolic syndrome.

To identify potent SCD1 selective inhibitors, novel assays were developed for high throughput screening and selectivity profiling. Lead optimisation led to highly potent (IC50 < 50nM) and selective SCD1 inhibitors. Profiling against a panel of over 100 assays indicate that these compounds do not inhibit other fatty acid desaturases, lipid metabolic enzymes, kinases, channels, receptors or transporters. ADMET analysis demonstrates that these SCD1 inhibitors possess favorable drug-like properties: high permeability, metabolic stability, no CYP450 inhibition or hERG blocking and acceptable pharmacokinetics. At an oral dose of 1mg/kg (twice daily) the inhibitors lower SCD1 activity in the fatty Zucker (*fa/fa*) rat by 85-89% and reduce the ratio of plasma 18:1n9/18:0 by 38-44%. After 4 weeks administration there was a statistically significant 15-26% ($p=0.0001$) lowering in body weight gain and 15-27% ($p=0.0001$) reduction in visceral fat weight. The reduction in body weight was not due to behavioural changes, taste aversion or reduced food consumption. These selective and safe small molecule SCD1 inhibitors duplicate the beneficial changes in obesity established in animal knockout studies and support their therapeutic application in the treatment of human obesity and metabolic syndrome.

For Author Duality of Interest Information, See Page A247.